CAGGGTCTCCCTTGCAACCTGAGGAGAGGTGCACACACTCTGAGGACCTAGGTGTGCAACCTCTGCCAGATGTGGG
GCGTGGCTACCCAGAGGCATGCCCCTCACCCAGCTCCACTGTCCCCACCTGCTGCTGCTGCTGTTGGTGCTGTCAT
GTCTGCCAGAGGCACCCTCTGCCCAGGTAATGGACTTTTTGTTTGAGAAGTGGAAGCTCTATAGTGACCAATGTCA
CCACAACCTAAGCCTGCTGCCCCCACCTACTGAGCTGGTCTGTAACAGAACCTTCGACAACTACTCCTGCTGGCCT
GACACCCCTCCCAACACCACTGCCAACATTTCCTGCCCCTGGTACCTACCTTGGTGCCACAAAGTGCAGCACCGCC
TAGTGTTCAAGAGGTGTGGGCCCGATGGGCAGTGGGTTCGAGGGCCACGGGGGCAGCCGTGGCGCAACGCCTCCCA
ATGTCAGTTGGATGATGAAGAGATCGAGGTCCAGAAGGGGGTGGCCAAGATGTATAGCAGCCAGCAGGTGATGTAC
ACCGTGGGCTACAGTCTGTCCCTGGGGGCCTTGCTCCTTGCGCTGGTCATCCTGCTGGGCCTCAGGAAGCTGCACT
GCACCCGAAACTACATCCATGGGAACCTGTTTGCGTCCTTTGTGCTCAAGGCTGGCTCTGTGTTGGTCATCGATTG
GCTGCTGAAGACACGGTACAGCCAGAAGATTGGCGATGACCTCAGTGTGAGCGTCTGGCTCAGTGACGGGGCGATG
GCCGGCTGCAGAGTGGCCACAGTGATCATGCAGTACGGCATCATACCCAACTATTGCTGGTTGCTGGTAGAGGGCG
TGTACCTGTACAGCCTGCTGAGCCTTGCCACCTTCTCTGAGAGGAGCTTCTTTTCCCTCTACCTGGGCATTGGCTG
GGGTGCGCCCCTGCTGTTTGTCATCCCCTGGGTGGTGGTCAAGTGTCTGTTTGAGAATGTTCAGTGCTGGACCAGC
AATGACAACATGGGATTCTGGTGGATCCTGCGTATTCCTGTCTTCCTGGCCTTACTGATCAATTTTTTCATCTTTG
TCCACATCATTCAACTTCTTGTGGCCAAGCTGCGTGCCCATCAGATGCACTATGCTGATTACAAGTTCCGGCTGGC
CAGGTCCACGCTGACCCTCATCCCTCTGCTGGGGGTCCACGAGGTGGTCTTTGCCTTTGTGACTGACGAGCATGCC
CAAGGCACCCTGCGCTCCACCAAGCTCTTTTTTGACCTGTTCCTCAGCTCCTTCCAGGGTCTGCTGGTGGCTGTTC
TCTACTGTTTCCTCAACAAGGAGGTGCAGGCAGAGCTGATGCGGCGTTGGAGGCAATGGCAAGAAGGCAAAGCTCT
TCAGGAGGAAAGGTTGGCCAGCAGCCATGGCAGCCACATGGCCCCAGCAGGGCCTTGTCATGGTGATCCCTGTGAG
AAACTTCAGCTTATGAGTGCAGGCAGCAGCAGTGGGACTGGCTGTGTGCCCTCTATGGAGACCTCGCTGGCCAGTA
GTCTCCCAAGGTTGGCTGACAGCCCCACCTGAATCTCCACTTGGAGCCTAGGCAGGTTGTGTTCAAGAAAGGGCCT
CAGAGGACAACCCAGAGCCAGATGCCCGGCCAAGGTTGAAGAGCCAAAGCAGCAAGACAGCAGCTTGTACTGTGCA
CACTCCCCTAACCTGTCCTAGCCTGGCACAGGCCACAGTGACAGAGTAGGGGTTGGATATGATGGAGAAGCCATGT
TATCTATGAACTCTGAGTGTTCCCATGTGTGTTGACATGGTCCCTGTACCCAGATATGTCCTTCAGTAAAAAGCTC
GAGTGGAGCTGCTGCACAGCTCGTGGACAGCAGGCTTGAAGCCCCCAGGGACGGGGTTTGGGAGGCCGGGGATGAG
CAGCACACTCAGCAGGTGGAGCGCTAGTGCAACCCAGGAAAGAA (SEQ ID NO:1)

FIGURE 1A

MPLTQLHCPHLLLLLLVLSCLPEAPSAQVMDFLFEKWKLYSDQCHHNLSLLPPPTELVCNRTFDNYSCWPDTPPNT
TANISCPWYLPWCHKVQHRLVFKRCGPDGQWVRGPRGQPWRNASQCQLDDEEIEVQKGVAKMYSSQQVMYTVGYSL
SLGALLLALVILLGLRKLHCTRNYIHGNLFASFVLKAGSVLVIDWLLKTRYSQKIGDDLSVSVWLSDGAMAGCRVA
TVIMQYGIIPNYCWLLVEGVYLYSLLSLATFSERSFFSLYLGIGWGAPLLFVIPWVVVKCLFENVQCWTSNDNMGF
WWILRIPVFLALLINFFIFVHIIQLLVAKLRAHQMHYADYKFRLARSTLTLIPLLGVHEVVFAFVTDEHAQGTLRS
TKLFFDLFLSSFQGLLVAVLYCFLNKEVQAELMRRWRQWQEGKALQEERLASSHGSHMAPAGPCHGDPCEKLQLMS
AGSSSGTGCVPSMETSLASSLPRLADSPT (SEQ ID NO:2)

FIGURE 1B

<u>underlined</u> = deleted in targeting construct

bold = sequence flanking Neo insert in targeting construct

CAGGGTCTCCCTTGCAACCTGAGGAGAGGTGCACACACTCTGAGGACCTAGGTGTGCAAC
CTCTGCCAGATGTGGGGCGTGGCTACCCAGAGGCATGCCCCTCACCCAGCTCCACTGTCC
CCACCTGCTGCTGCTGCTGTTGGTGCTGTCATGTCTGCCAGAGGCACCCTCTGCCCAGGT
AATGGACTTTTTGTTTGAGAAGTGGAAGCTCTATAGTGACCAATGTCACCACAACCTAAG
CCTGCTGCCCCCACCTACTGAGCTGGTCTGTAACAGAACCTTCGACAACTACTCCTGCTG
GCCTGACACCCCTCCCAACACCACTGCCAACATTTCCTGCCCCTGGTACCTACCTTGGTG
CCACAAAGTGCAGCACCGCCTAGTGTTCAAGAGGTGTGGGCCCGATGGGCAGTGGGTTCG
AGGGCCACGGGGGCAGCCGTGGCGCAACGCCTCCCAATGTCAGTTGGATGATGAAGAGAT
CGAGGTCCA**GAAGGGGGTGGCCAAGATGTATAGCAGCCAGCAGGTGATGTACACCGT**<u>GGG
CTACAGTCTGTCCCTGGGGGCCTTGCTCCTTGCGCTGGTCATCCTGCTGGGCCTCAGGAA
GCTGCACTGCACCCGAAACTACATCCATGGGAACCTGTTTGCGTCCTTTGTGCTCAAGGC
TGGCTCTGTGTTGGTCATCGATTGGCTGCTGAAGACACGGTACAGCCAGAAGATTGGCGA
TGACCTCAGTGTGAGCGTCTGGCTCAGTGACGGGGCGATGGCCGGCTGCAGAGTGGCCAC
AGTGATCATGCAGTACGGCATCATACCCAACTATTGCTGGTTGCTGGTAGAGGGCGTGTA
CCTGTACAGCCTGCTGAGCCTTGCCACCTTCTCTGAGAGGAGCTTCTTTTCCCTCTACCT
GGGCATTGGCTGGGGTGCGCCCCTGCTGTTTGTCATCCCCTGGGTGGTGGTCAAGTGTCT
GTTTGAGAATGTTCAGTGCTGGACCAGCAATGACAACATGGGATTCTGGTGGATCCTGCG
TATTCCTGTCTTCCTGGCCTTACTGATCAATTTTTTCATCTTTGTCCACATCATTCAACT
TCTTGTGGCCAAGCTGCGTGCCCATCAGATGCACTATGCTGATTACAAGTTCCGGCTGGC
CAGGTCCACGCTGACCCTCATCCCTCTGCTGGGGGTCCAC**GAGGTGGTCTTTGCCTTTGT**
GACTGACGAGCATGCCCAAGGCACCCTGCGCTCCACCAAGCTCTTTTTTGACCTGTTCCT
CAGCTCCTTCCAGGGTCTGCTGGTGGCTGTTCTCTACTGTTTCCTCAACAAGGAGGTGCA
GGCAGAGCTGATGCGGCGTTGGAGGCAATGGCAAGAAGGCAAAGCTCTTCAGGAGGAAAG
GTTGGCCAGCAGCCATGGCAGCCACATGGCCCCAGCAGGGCCTTGTCATGGTGATCCCTG
TGAGAAACTTCAGCTTATGAGTGCAGGCAGCAGCAGTGGGACTGGCTGTGTGCCCTCTAT
GGAGACCTCGCTGGCCAGTAGTCTCCCAAGGTTGGCTGACAGCCCCACCTGAATCTCCAC
TTGGAGCCTAGGCAGGTTGTGTTCAAGAAAGGGCCTCAGAGGACAACCCAGAGCCAGATG
CCCGGCCAAGGTTGAAGAGCCAAAGCAGCAAGACAGCAGCTTGTACTGTGCACACTCCCC
TAACCTGTCCTAGCCTGGCACAGGCCACAGTGACAGAGTAGGGGTTGGATATGATGGAGA
AGCCATGTTATCTATGAACTCTGAGTGTTCCCATGTGTGTTGACATGGTCCCTGTACCCA
GATATGTCCTTCAGTAAAAAGCTCGAGTGGAGCTGCTGCACAGCTCGTGGACAGCAGGCT
TGAAGCCCCCAGGGACGGGGTTTGGGAGGCCGGGGATGAGCAGCACACTCAGCAGGTGGA
GCGCTAGTGCAACCCAGGAAAGAA

FIGURE 2A

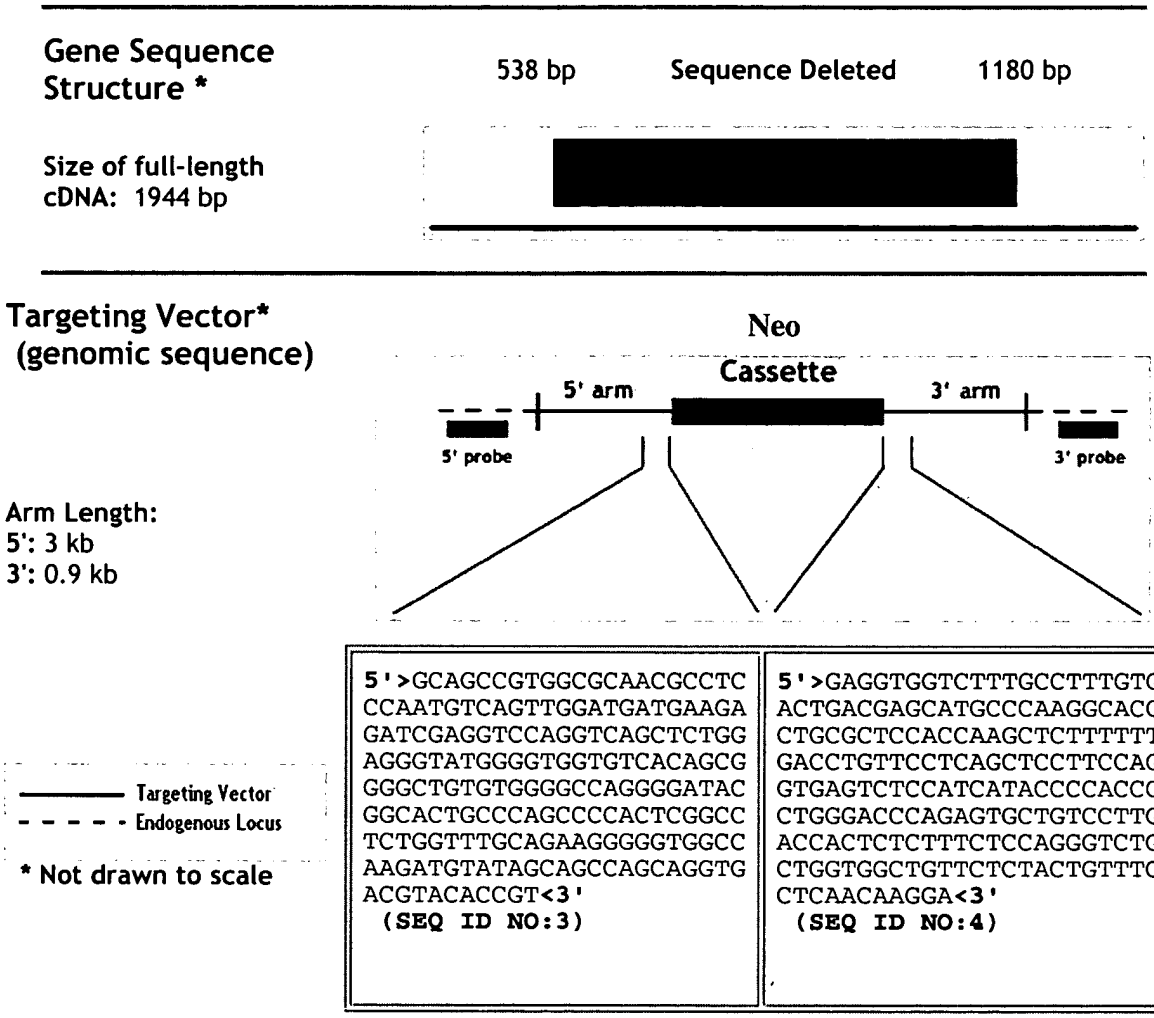
Gene Sequence Structure *
538 bp
Sequence Deleted
1180 bp
Size of full-length cDNA: 1944 bp
Targeting Vector* (genomic sequence)
Neo
Cassette
5' arm
3' arm
5' probe
3' probe
Arm Length:
5': 3 kb
3': 0.9 kb
Targeting Vector
Endogenous Locus
* Not drawn to scale
5'>GCAGCCGTGGCGCAACGCCTC
CCAATGTCAGTTGGATGATGAAGA
GATCGAGGTCCAGGTCAGCTCTGG
AGGGTATGGGGTGGTGTCACAGCG
GGGCTGTGTGGGGCCAGGGGATAC
GGCACTGCCCAGCCCCACTCGGCC
TCTGGTTTGCAGAAGGGGGTGGCC
AAGATGTATAGCAGCCAGCAGGTG
ACGTACACCGT<3'
(SEQ ID NO:3)
5'>GAGGTGGTCTTTGCCTTTGTG
ACTGACGAGCATGCCCAAGGCACC
CTGCGCTCCACCAAGCTCTTTTTT
GACCTGTTCCTCAGCTCCTTCCAG
GTGAGTCTCCATCATACCCCACCC
CTGGGACCCAGAGTGCTGTCCTTG
ACCACTCTCTTTCTCCAGGGTCTG
CTGGTGGCTGTTCTCTACTGTTTC
CTCAACAAGGA<3'
(SEQ ID NO:4)

FIGURE 2B

GGATCTGGCAGCGCCGCGAAGACGAGCGGTCACCGGCGCCCGACCCGAGCGCGCCCAGAGGACGGCGGGGAGCCAA
GCCGACCCCCGAGCAGCGCCGCGCGGGCCCTGAGGCTCAAAGGGGCAGCTTCAGGGGAGGACACCCCACTGGCCAG
GACGCCCCAGGCTCTGCTGCTCTGCCACTCAGCTGCCCTCGGAGGAGCGTACACACACACCAGGACTGCATTGCCC
CAGTGTGCAGCCCCTGCCAGATGTGGGAGGCAGCTAGCTGCCCAGAGGCATGCCCCCCTGCCAGCCACAGCGACCC
CTGCTGCTGTTGCTGCTGCTGCTGGCCTGCCAGCCACAGGTCCCCTCCGCTCAGGTGATGGACTTCCTGTTTGAGA
AGTGGAAGCTCTACGGTGACCAGTGTCACCACAACCTGAGCCTGCTGCCCCCTCCCACGGAGCTGGTGTGCAACAG
AACCTTCGACAAGTATTCCTGCTGGCCGGACACCCCCGCCAATACCACGGCCAACATCTCCTGCCCCTGGTACCTG
CCTTGGCACCACAAAGTGCAACACCGCTTCGTGTTCAAGAGATGCGGGCCCGACGGTCAGTGGGTGCGTGGACCCC
GGGGGCAGCCTTGGCGTGATGCCTCCCAGTGCCAGATGGATGGCGAGGAGATTGAGGTCCAGAAGGAGGTGGCCAA
GATGTACAGCAGCTTCCAGGTGATGTACACAGTGGGCTACAGCCTGTCCCTGGGGGCCCTGCTCCTCGCCTTGGCC
ATCCTGGGGGGCCTCAGCAAGCTGCACTGCACCCGCAATGCCATCCACGCGAATCTGTTTGCGTCCTTCGTGCTGA
AAGCCAGCTCCGTGCTGGTCATTGATGGGCTGCTCAGGACCCGCTACAGCCAGAAAATTGGCGACGACCTCAGTGT
CAGCACCTGGCTCAGTGATGGAGCGGTGGCTGGCTGCCGTGTGGCCGCGGTGTTCATGCAATATGGCATCGTGGCC
AACTACTGCTGGCTGCTGGTGGAGGGCCTGTACCTGCACAACCTGCTGGGCCTGGCCACCCTCCCCGAGAGGAGCT
TCTTCAGCCTCTACCTGGGCATCGGCTGGGGTGCCCCCATGCTGTTCGTCGTCCCCTGGGCAGTGGTCAAGTGTCT
GTTCGAGAACGTCCAGTGCTGGACCAGCAATGACAACATGGGCTTCTGGTGGATCCTGCGGTTCCCCGTCTTCCTG
GCCATCCTGATCAACTTCTTCATCTTCGTCCGCATCGTTCAGCTGCTCGTGGCCAAGCTGCGGGCACGGCAGATGC
ACCACACAGACTACAAGTTCCGGCTGGCCAAGTCCACGCTGACCCTCATCCCTCTGCTGGGCGTCCACGAAGTGGT
CTTTGCCTTCGTGACGGACGAGCACGCCCAGGGCACCCTGCGCTCCGCCAAGCTCTTCTTCGACCTCTTCCTCAGC
TCCTTCCAGGGCCTGCTGGTGGCTGTCCTCTACTGCTTCCTCAACAAGGAGGTGCAGTCGGAGCTGCGGCGGCGTT
GGCACCGCTGGCGCCTGGGCAAAGTGCTATGGGAGGAGCGGAACACCAGCAACCACAGGGCCTCATCTTCGCCCGG
CCACGGCCCTCCCAGCAAGGAGCTGCAGTTTGGGAGGGGTGGTGGCAGCCAGGATTCATCTGCGGAGACCCCCTTG
GCTGGTGGCCTCCCTAGATTGGCTGAGAGCCCCTTCTGAACCCTGCTGGGACCCCAGCTAGGGCTGGACTCTGGCA
CCCAGAGGCGTCGCTGGACAACCCAGAACTGGACGCCCAGCTGAGGCTGGGGGCGGGGGAGCCAACAGCAGCCCCC
ACCTACCCCCCACCCCCAGTGTGGCTGTCTGCGAGATTGGGCCTCCTCTCCCTGCACCTGCCTTGTCCCTGGTGCA
GAGGTGAGCAGAGGAGTCCAGGGCGGGAGTGGGGGCTGTGCCGTGAACTGCGTGCCAGTGTCCCCACGTATGTCGG
CACGTCCCATGTGCATGGAAATGTCCTCCAACAATAAAGAGCTCAAGTGGTCACCGTG (SEQ ID NO:5)

FIGURE 3A

MPPCQPQRPLLLLLLLLACQPQVPSAQVMDFLFEKWKLYGDQCHHNLSLLPPPTELVCNRTFDKYSCWPDTPANTT
ANISCPWYLPWHHKVQHRFVFKRCGPDGQWVRGPRGQPWRDASQCQMDGEEIEVQKEVAKMYSSFQVMYTVGYSLS
LGALLLALAILGGLSKLHCTRNAIHANLFASFVLKASSVLVIDGLLRTRYSQKIGDDLSVSTWLSDGAVAGCRVAA
VFMQYGIVANYCWLLVEGLYLHNLLGLATLPERSFFSLYLGIGWGAPMLFVVPWAVVKCLFENVQCWTSNDNMGFW
WILRFPVFLAILINFFIFVRIVQLLVAKLRARQMHHTDYKFRLAKSTLTLIPLLGVHEVVFAFVTDEHAQGTLRSA
KLFFDLFLSSFQGLLVAVLYCFLNKEVQSELRRRWHRWRLGKVLWEERNTSNHRASSSPGHGPPSKELQFGRGGGS
QDSSAETPLAGGLPRLAESPF (SEQ ID NO:6)

FIGURE 3B

Fasting Whole Blood Glucose Results

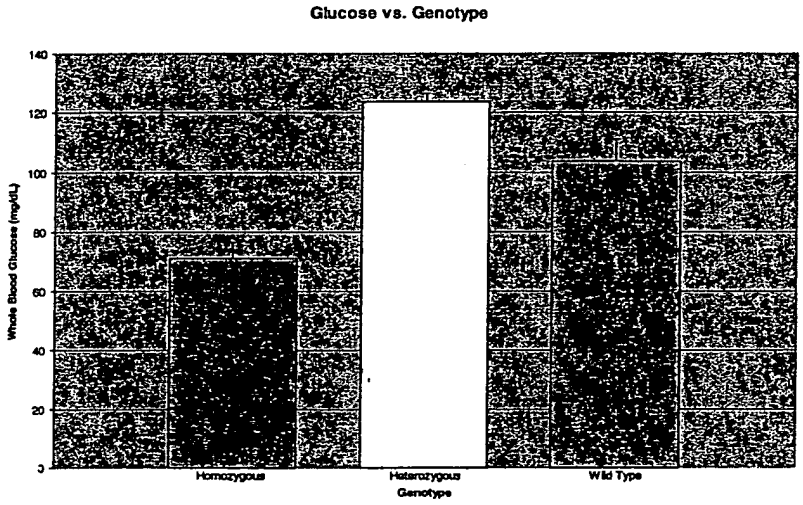

FIGURE 4

Relationship of Blood Glucose Levels to Severity of Pancreatic Lesions

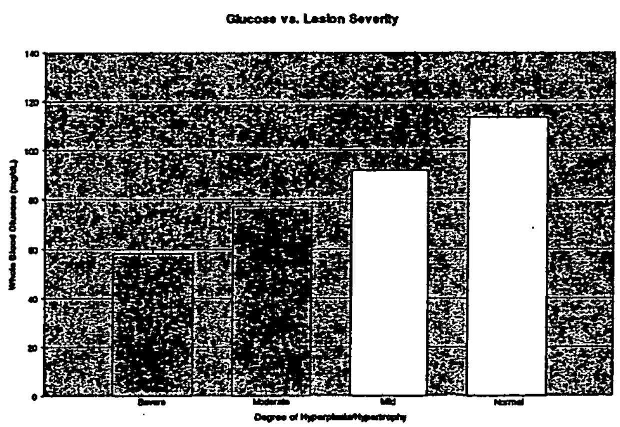

FIGURE 5

Serum Insulin Level Results

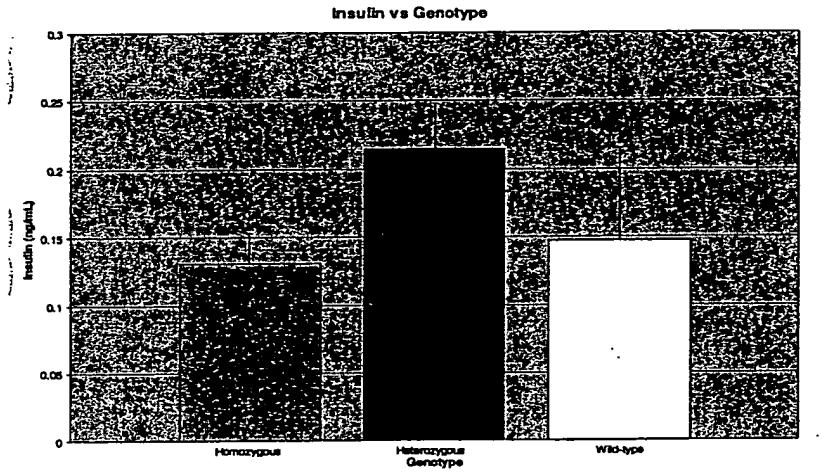

FIGURE 6

Relationship of Serum Insulin Levels to Pancreatic Lesion Severity

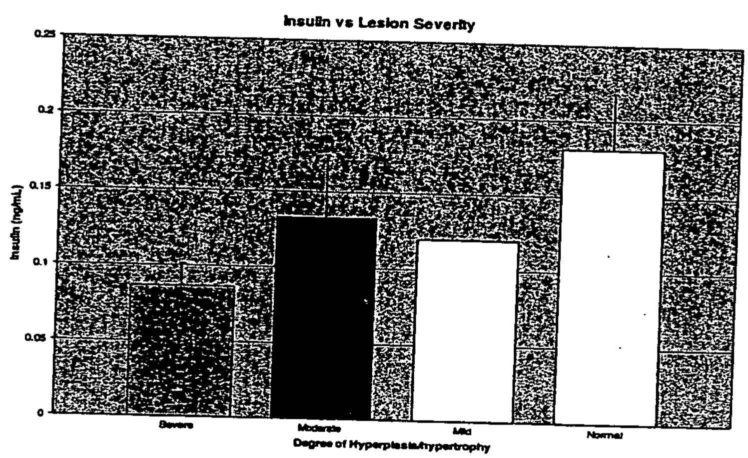

FIGURE 7

Serum Glucagon Results

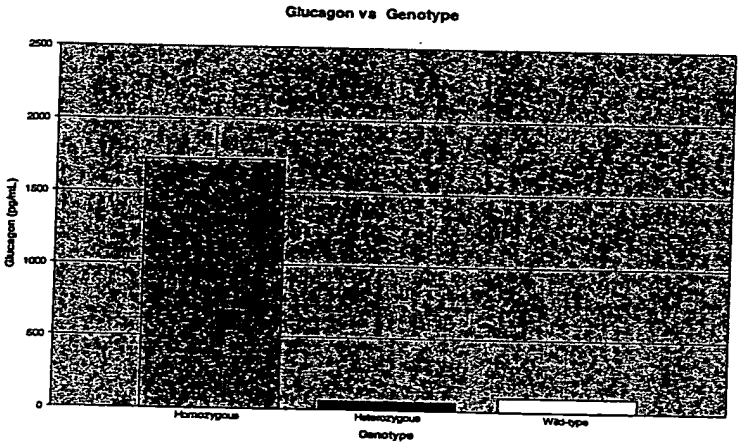

FIGURE 8

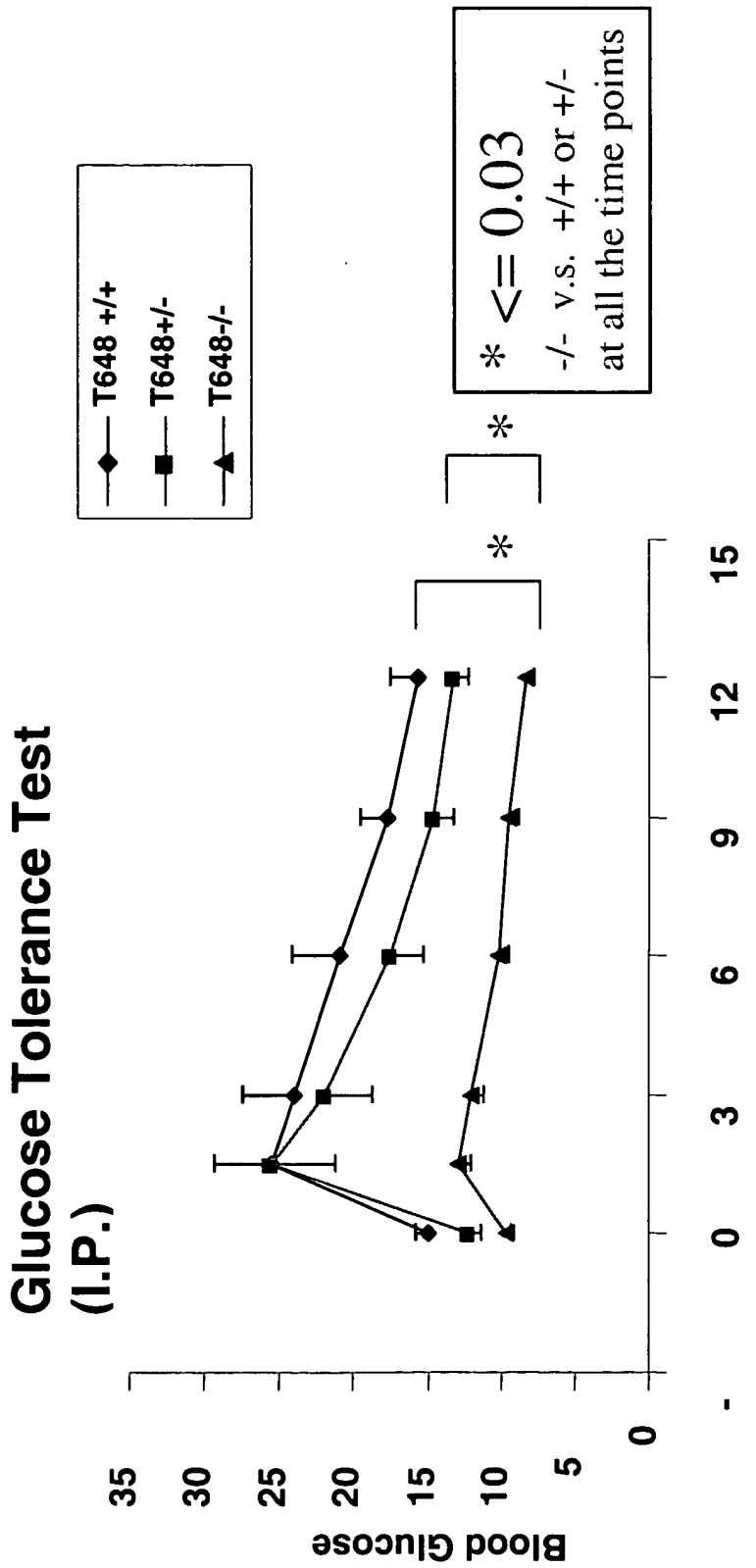
Glucose Tolerance Test
(I.P.)
T648 +/+
T648+/-
T648-/-
* <= 0.03
-/- v.s. +/+ or +/-
at all the time points
*
*
Blood Glucose
35
30
25
20
15
10
5
0
-
0
3
6
9
12
15
Minutes After Glucose

FIGURE 9

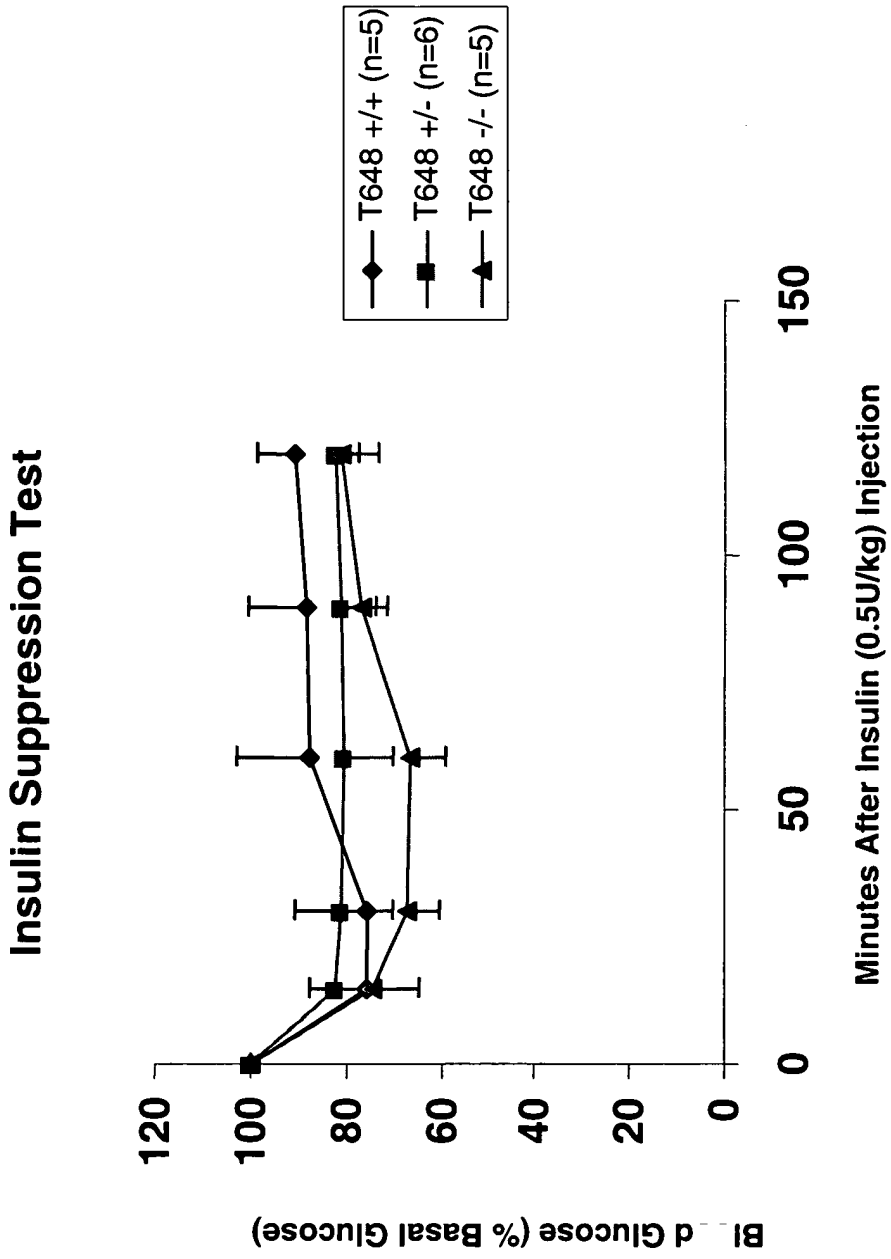
Insulin Suppression Test
T648 +/+ (n=5)
T648 +/- (n=6)
T648 -/- (n=5)
120
100
80
60
40
20
0
0
50
100
150
Minutes After Insulin (0.5U/kg) Injection
Bl__d Glucose (% Basal Glucose)

FIGURE 10

Glucose Stimulated Insulin Secretion Test

FIGURE 11

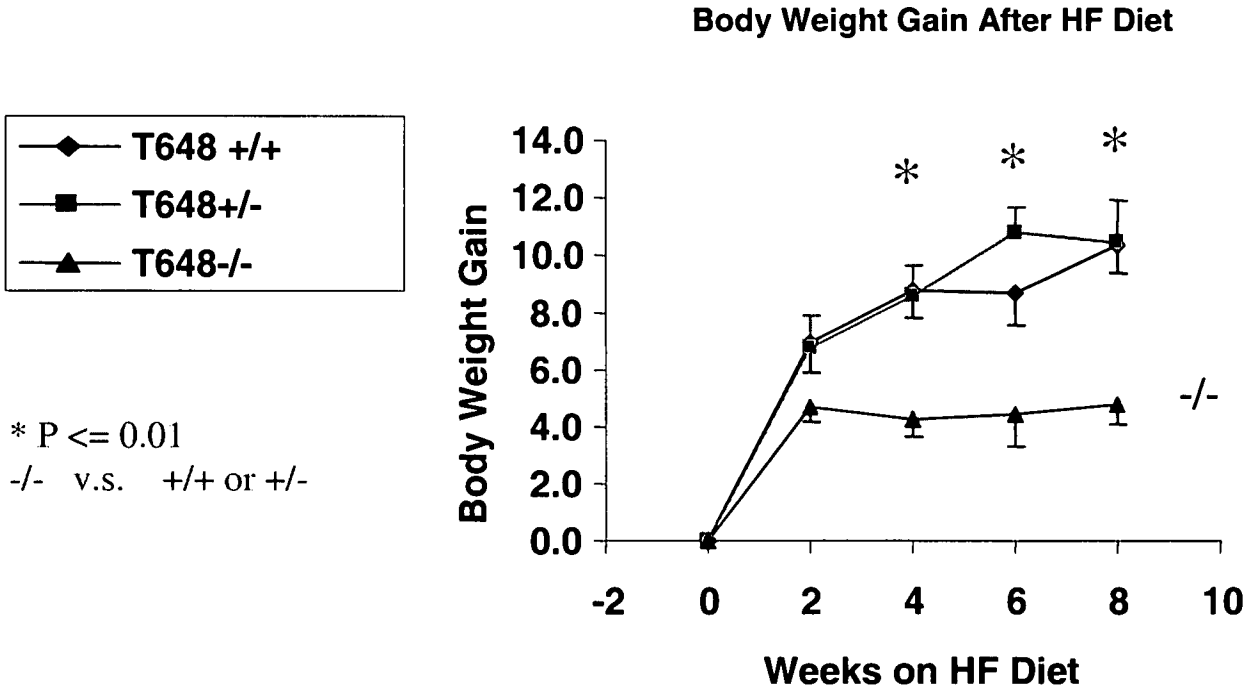
Body Weight Gain After HF Diet
T648 +/+
T648+/-
T648-/-
* P <= 0.01
-/- v.s. +/+ or +/-
Body Weight Gain
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
-2
0
2
4
6
8
10
Weeks on HF Diet
*
*
*
-/-

FIGURE 12

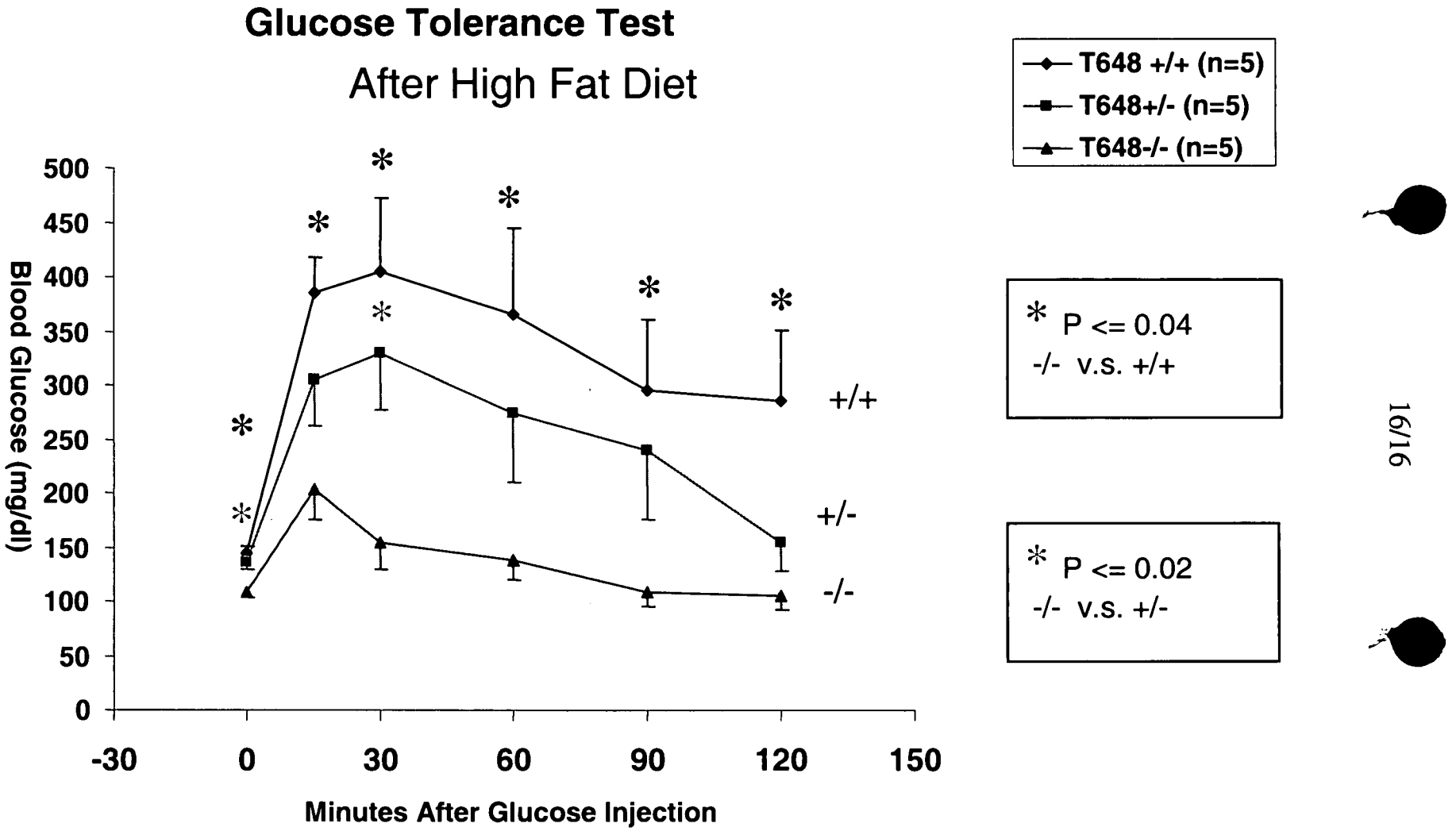
Glucose Tolerance Test
After High Fat Diet
T648 +/+ (n=5)
T648+/- (n=5)
T648-/- (n=5)
* P <= 0.04
-/- v.s. +/+
* P <= 0.02
-/- v.s. +/-
Blood Glucose (mg/dl)
500
450
400
350
300
250
200
150
100
50
0
-30
0
30
60
90
120
150
Minutes After Glucose Injection
+/+
+/-
-/-

FIGURE 13